CLINICAL EVALUATION OF AN EPIGENETIC ASSAY TO PREDICT MISSED CANCER IN PROSTATE BIOPSY SPECIMENS

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ABSTRACT

Approximately 1 million prostate biopsies are performed yearly in the United States, with only ~25% resulting in prostate cancer diagnosis. However, ~40% of men receive multiple biopsies for fear of cancer being missed. DNA hypermethylation is ideally suited for early disease detection and could be used to prevent unnecessary biopsies. Men with low-risk epigenetic signatures may forego subsequent biopsy and potential complications. A meta-analysis of two validation studies was conducted to gain additional insight into the benefits for patient risk stratification. In the Methylation Analysis to Locate Occult Cancer (MATLOC) study a negative predictive value of 90% was obtained, which represents a significant improvement over standard of care. This was confirmed in the Detection of Cancer Using Methylated Events in Negative Tissue (DOCUMENT) study (88% negative predictive value), which was designed to validate the performance in an independent cohort. The epigenetic assay, in combination with other known risk factors, may help reduce unnecessary repeat prostate biopsies and identify men at highest risk of harboring occult high-grade prostate cancer.

INTRODUCTION

Prostate cancer (PCa) is not only the most common cancer in men, but also the tumor that is affected most by the use of molecular markers. The serum prostate-specific antigen (PSA) has been used since the mid-1980s as a screening and diagnostic marker (1). However, PSA is not cancer specific, and it often exhibits elevated serum concentrations

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in men with benign conditions or, conversely, levels in the normal range for men harboring PCa (2,3). More recently, this has elicited a large controversy for this marker, with two large studies indicating that PSA has no or a minor effect on decreasing PCa death (4,5). In addition, it is expected that as a result of PSA screening, many men were over-treated for insignificant disease (6,7). This has brought about new dilemmas, centered on two major topics.

First, when risk factors, including PSA, indicate that a man has an increased chance of harboring PCa, a prostate biopsy is often performed. A typical biopsy samples less than 1% of a man's prostate, albeit in well-chosen, dispersed locations, resulting in a potential sampling error and false negative diagnoses (8). When risk factors persist, it could be due to a false negative index biopsy because of sampling of a non-cancer–related region or because of a non-PCa–related reason. Hence, the question arises about who should undergo a repeat biopsy and who could forego an unnecessary invasive procedure, since this brings about unwanted side effects and risks (9,10).

Second, when cancer is found, it is often low grade, in an early stage, and can be considered indolent or insignificant. To this extent, the National Comprehensive Cancer Network has developed criteria indicating the risk of having aggressive disease based on a patient's clinical characteristics (11). Similarly the Prostate Cancer Prevention Trial (PCPT) risk calculator (RC) aims to achieve the same objective, that is, to estimate a man's risk of harboring aggressive disease based on past experience for men with certain clinical and demographic characteristics (i.e., race, age, PSA, family history, digital rectal examination [DRE], and outcome of potential previous biopsy specimens) (12,13). However, recent studies have indicated that a significant proportion of men who were initially considered for active surveillance eventually showed symptoms of more aggressive disease and eventually underwent more radical treatment (14–18). This results from the systematic biopsy sampling error in combination with the lack of cancer specificity of PSA.

Epigenetic mechanisms lie at the basis of creating the plethora of distinct cell types and cellular functions in the human body, with DNA methylation being the most studied and best understood epigenetic event (19). In general, when the promoter region of a gene becomes hypermethylated, the expression of this gene is shut down (20). Although this type of regulation is necessary and wanted in all human cells, these processes are aberrantly altered in tumor cells (21). In particular when compared to the well-known DNA mutations, epigenetic

aberrations are the most frequent and abundant alterations in the oncogenic process, transforming a normal cell into a tumor cell (22–24). Detecting these aberrant DNA-methylation events is therefore ideally suited for biomarker purposes, especially because certain markers can be detected in a cancer-specific manner from the earliest stages of the oncogenic process (25,26).

DNA-methylation events in PCa have also been extensively described. The aberrant silencing of the DNA detoxifying gene *GSTP1* in particular has been shown to be a sensitive cancer-specific biomarker, occurring in 80% to 90% of all prostate tumors (26). This gene has been thoroughly studied since the mid-1990s, resulting in a firm body of evidence. Additional genes have been identified as good complementary diagnostic and prognostic markers for PCa (26–28). Most notably, a DNA-methylation assay has been described to identify who can likely forego an unnecessary repeat biopsy (29,30). The genes involved in this assay are *GSTP1*, *RASSF1,* and *APC*, with their main strength being the fact that DNA methylation of these genes can be detected in a tumor-associated field. These molecular alterations can be observed in normal-appearing tissue adjacent to a prostate tumor, a phenomenon called the field effect (31); therefore, DNA methylation can overcome, to some extent, the biopsy sampling error (29,30).

Currently, molecular markers are under scrutiny, in part because of PSA and the unrealistic expectations for novel molecular markers. Because PSA is not sufficiently specific for high-grade or aggressive PCa, it has led to a high degree of over-diagnosis; however, it also has a modest contribution to the decrease in prostate cancer death (4,5,32). To better weigh this benefit for one individual versus the benefit/downside for an entire population, PSA could be used in combination with other molecular markers or risk factors to improve the diagnosis. Similar observations can be made for novel molecular markers, that is, if these offer significant improvement over the current clinical practice, a method can be identified to best incorporate these to enhance overall patient management. None of the current or even future methods will be perfect, but the reference should be whether a significant enhancement can be made at the level of the individual patient, of course when factoring in the cost of implementing this new diagnostic workflow. A model that combines the information of novel molecular markers (in particular epigenetic profiling) with traditional risk factors into a clinically useful risk score could not only improve, but also objectify patient management, leveraging the strengths of all parameters involved.

MATERIALS AND METHODS

A meta-analysis was performed on two previously published cohorts that were combined into one set of 803 patients, 483 European men from the Methylation Analysis to Locate Occult Cancer (MATLOC) study and 349 US men from the Detection of Cancer Using Methylated Events in Negative Tissue (DOCUMENT) study (29,30). The observations regarding DNA methylation and the associations with the other risk factors were similar in both cohorts. All of these men had PCa-negative index biopsy specimens, which were profiled using an epigenetic assay (ConfirmMDx for Prostate Cancer; MDxHealth, Irvine, California). This epigenetic assay is a multiplex methylation-specific polymerase chain reaction assay detecting methylation of *GTSP1*, *RASSF1,* and *APC,* and was used to evaluate all individual core biopsy tissues. All these men have had a repeat biopsy within 30 months of their index biopsy because of persistent risk factors (PSA, DRE, or family history), resulting in a PCa diagnosis for 179 (22.3%) of the men. Men with atypia detected on the original biopsy were not included because they were routinely re-biopsied. However, men with benign pathology that had atypia identified upon central pathology review were allowed. Because the general cohort was enriched for men with a cancer-positive repeat biopsy (DOCUMENT), the cancer prevalence was adjusted to 18% based on an estimate in a natural history cohort (MATLOC) for prevalence-dependent metrics such as the negative predictive value (NPV), which was calculated based on accurate sensitivity and specificity estimates. This adjustment can be made when robust estimates of both sensitivity and specificity, both of which are prevalence-independent, and the actual PCa prevalence in a biopsy population are available. A detailed overview of the most relevant clinical and demographic characteristics is given in Table 1.

All analyses were performed in R (33). Continuous variables were compared with Welch's t-test or the Mann-Whitney-Wilcoxon test in case of deviation of normality, and categorical variables with Pearson's chi-squared test with Yates' continuity correction. Proportions were compared to each other or reference values using a binomial test. The R library pROC was used for area under the curve (AUC) of the receiver-operating characteristic calculations (34). This metric was used as a measure for the overall performance of continuous risk scores and logistic regression models. The outcome, that is, the chance of having no, low-grade, or high-grade PCa detected, of the PCPT RC was also calculated using the R code of version 2 of the RC (13).

TABLE 1.
Main Clinical and Demographic Characteristics of the MATLOC and DOCUMENT Cohorts

		All Patients	Cases	Controls	*P* Value
Clinical centers	All Centers	*803*	179 (22.3)	*624*	
	Edinburgh	387	71 (18.3)	316	
	Belgium	96	16 (16.7)	80	
	Cleveland Clinic	70	21 (30)	49	
	EVMS	50	15 (30)	35	
	Johns Hopkins	67	16 (23.9)	51	
	Lahey Clinic	71	24 (33.8)	47	
	UCLA	62	16 (25.8)	46	
Age, y	Mean	62.9	64.1	62.5	0.009
	Median (IQR)	63 (59.0-67.5)	65 (59.0-69.0)	62.0 (58.0-67.0)	
Race	Caucasian	646 (80.5)	134 (75.3)	512 (82.1)	0.042
PSA (ng/ml)	Mean	7.0	7.5	6.85	0.209
	Median (IQR)	5.6 (4.2-8.2)	5.5 (4.1-8.6)	5.6 (4.2-8.1)	
DRE	Abnormal	198 (31.8)	46 (33.6)	152 (31.3)	0.695
Pathology of index biopsy	Benign	538 (67.0)	98 (54.7)	440 (70.5)	<0.001
	HGPIN	193 (24.0)	51 (28.5)	142 (22.8)	
	Atypia	72 (9.0)	30 (16.8)	42 (6.7)	
Time between biopsies (mo)	Mean	12.1	10.7	12.5	0.819
	Median (IQR)	9.4 (3.7-16.9)	10.4 (4.3-15.4)	9.2 (3.5-17.7)	
GS	Low (≤ 6)		106 (61.3)		
	High (≥ 7)		67 (38.7)		

Abbreviations: MATLOC, Methylation Analysis to Locate Occult Cancer; DOCUMENT, Detection of Cancer Using Methylated Events in Negative Tissue; EVMS, Eastern Virginia Medical School; UCLA, University of California – Los Angeles; PSA, prostate-specific antigen; IQR, interquartile ratio; DRE, digital rectal examination; HGPIN, high-grade prostatic intraepithelial neoplasia; GS, Gleason score.

RESULTS

Epigenetic Assay to Avoid Unnecessary Repeat Biopsies

The previously determined cutoffs for *GSTP1*, *RASSF1,* and *APC* were applied to the entire cohort, resulting in 116 methylation-positive men of 179 men with a PCa-positive repeat biopsy specimen and 398 methylation-negative men of 624 men with a PCa-negative repeat biopsy specimen. The sensitivity and specificity of the assay were 64.8% and 63.8%, respectively, and overall an NPV of 89.2% was obtained, a significant increase over the NPV of standard of care (P <0.001). In this cohort, 77.7% of all repeat biopsies performed under standard of care were unnecessary, compared to 66.1% with the

epigenetic assay, which constitutes a significant reduction (P <0.001). Overall, if only methylation-positive men were re-biopsied, the biopsy burden would decrease to 42.6% relative to standard of care.

Performance for Occult High-Grade Cancer

Currently, PCa diagnostics often focus on significant or high-grade cancer. In the current cohort, 67 men were identified with a pathological Gleason score (GS) ≥7. The number of missed high-grade cancers by biopsy is reduced by 64.2% by use of the epigenetic assay (P <0.001). From Table 1 it can be derived that, in this population, 38.7% of the men diagnosed with PCa will have high-grade disease detected at time of biopsy. Hence, the overall prevalence of men with high-grade PCa in the general biopsy population can be estimated to be 7.0%, with an NPV of 96.0% for the epigenetic assay, which is significantly better than the standard of care (P = 0.004). In summary, approximately 7 of every 100 men with a PCa-negative biopsy specimen harbor occult high-grade disease. The epigenetic assay would result in a significant reduction to less than 3 men of every 100 with occult high-grade PCa (P <0.001).

In this cohort, only 15 men with GS ≥ 8 disease were detected at the time of repeat biopsy. Anecdotally, the sensitivity of the assay for these men increases further to 80%. A similarly high sensitivity of 78.3% was obtained for 23 men with negative index biopsy specimens, but who are at high risk (either GS ≥8 or PSA ≥20 ng/ml) according to the National Comprehensive Cancer Network guidelines based on the outcome of their repeat biopsy.

Improving Patient Management

The performance of the epigenetic assay was compared to the other risk factors assessed in this cohort at the time of the PCa-negative index biopsy, which ultimately led to the repeat biopsy. PSA values were log-transformed because this led to a generally better predictive value of this risk factor. In a univariate analysis, the epigenetic assay outperformed all other risk factors, with only the presence of atypia coming close to this result (Figure 1). Epigenetics has the additional advantage that it is an objective measurement, whereas atypia can be subjective, with inter-observer variability and reproducibility error. The epigenetic assay (P <0.001), the presence of atypia (P <0.001), or high-grade prostatic intraepithelial neoplasia (HGPIN; P = 0.016) and age (P = 0.008) were significant predictors of the outcome of a repeat biopsy, whereas PSA (P = 0.144) and DRE (P = 0.620) were not.

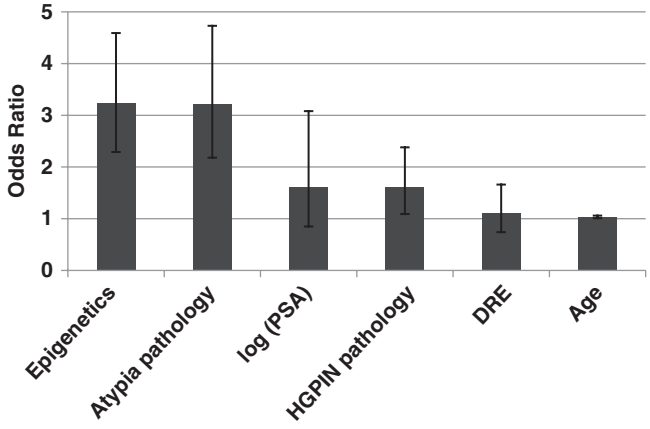

FIG. 1. Odds ratios for the univariate analyses of all risk factors that were assessed within the combined MATLOC and DOCUMENT cohorts. The error bars depict the 95% confidence intervals. PSA, prostate-specific antigen; DRE, digital rectal examination.

These risk factors were combined in a multivariate logistic regression model, indicating that both the presence of atypia (P <0.001) and the epigenetic assay (P <0.001) are significant, independent predictors (Figure 2). PSA exhibited a minor, borderline significant effect (P = 0.071), indicating that it might have a role when considered simultaneously with other risk factors. Finally, the presence of HGPIN in the index biopsy (P = 0.122), age (P = 0.232), and DRE (P = 0.846) did not contribute significantly in predicting the outcome of a repeat biopsy.

DNA-Methylation Intensity

Whereas the epigenetic assay is set up to produce binary results, that is, methylation-positive men are at increased risk of harboring occult (high-grade) cancer, versus methylation-negative men that can most likely forego an unnecessary repeat biopsy, the raw data allows assessment of the prostate's overall intensity of DNA-methylation aberrations. The methylation intensity is measured per core, normalizing the methylation levels above background for each one of the genes, and then averaged over all available cores. Methylation intensity was highly significant in a univariate analysis, resulting in an odds ratio (OR) of 16.23 (95% confidence interval [CI]: 6.71-39.26; P <0.001). Interestingly, methylation intensity appeared to be an independent predictor when compared to the binary outcome of the epigenetic assay. A multivariate logistic regression model indicated that both variables were significant

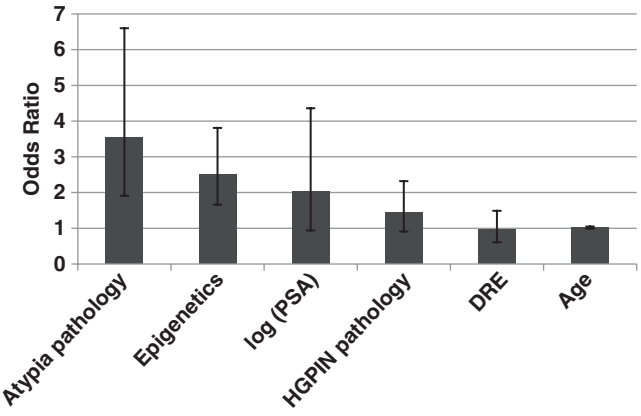

FIG. 2. Odds ratios for all the risk factors within the multivariate logistic regression model. The error bars depict the 95% confidence intervals. Only the presence of atypia and the epigenetic assay were significant in this model, with a borderline result for log (prostate-specific antigen). PSA, prostate-specific antigen; HGPIN, high-grade prostatic intraepithelial neoplasia; DRE, digital rectal examination.

predictors (both P <0.001) associated with ORs of 6.44 (95% CI: 2.57-16.13) and 2.37 (95% CI: 1.62-3.49), respectively. Although AUC is not ideally suited to assess the performance of binary variables, such as the epigenetic assay, it is a good tool to determine and compare the predictive power of continuous variables such as risk scores resulting from logistic regression models. Methylation intensity alone reached an AUC of 0.646 compared to 0.669 for the model combining the epigenetic assay and DNA-methylation intensity (Figure 3).

Molecular Markers Versus Traditional Risk Assessment

Combining risk factors into one model is expected to yield a better performance to assess patient risk of harboring occult PCa. The PCPT RC was used as benchmark for such a model. Because no data were available on %free PSA and familial history, the performance of these risk factors could not be assessed. The OR for the chance of finding either low-grade or high-grade PCa with the PCPT RC was 1.02 (95% CI: 1.00-1.04), making it a significant risk predictor (P = 0.020).

Logistic regression analysis indicated that epigenetics in general, and the epigenetic assay in particular, were the best-performing risk factors and hence would warrant inclusion in a multimodal risk assessment approach. This hypothesis was further strengthened because an AUC of 0.536 was obtained for PCPT RC, predicting the presence of

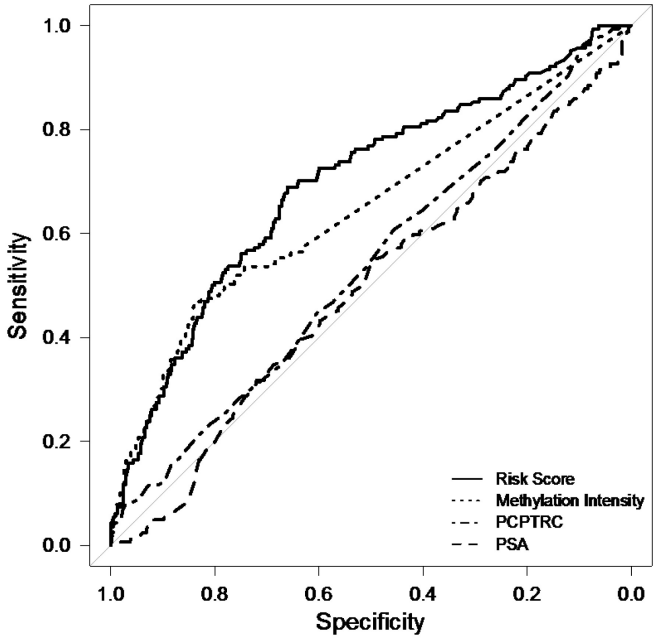

FIG. 3. Receiving operator characteristic curves for PSA, PCPT RC, DNA-methylation intensity, and a risk score as determined with an epigenetics-based multimodal risk assessment model for all (both high- and low-grade) prostate cancer. PSA, prostate-specific antigen; PCPT RC, Prostate Cancer Prevention Trial Risk Calculator.

both low-grade and high-grade cancers. DNA-methylation intensity by itself was already significantly better in predicting the presence of occult cancer ($P = 0.003$), hence, epigenetics will likely be central in a multimodal risk prediction model. The role of PSA as an individual risk factor was further weakened with a non-optimal patient stratification indicated by an AUC of 0.493 ($P = 0.357$ relative to PCPT RC and $P < 0.001$ relative to DNA-methylation intensity) (Figure 3).

Multimodal Risk Assessment Model for Repeat Biopsy Outcome

Whereas logistic regression analyses indicated that the epigenetic assay served as strongest, independent predictor, a multimodal patient risk assessment would further enhance patient management. When all risk factors, including the epigenetic assay (AUC = 0.643), PSA (AUC = 0.489), pathology (AUC = 0.581), DRE (AUC = 0.509), and age (AUC = 0.569) were included in one model, an AUC of 0.692 was obtained. DRE

and age were not statistically significant, and hence, could be excluded, whereas PSA was borderline (P = 0.074) and therefore retained in the model. This model, with justified contribution of all risk factors, reached an AUC of 0.681 and was significantly better than the PCPT RC (P <0.001). Similarly, a model can be built combining the epigenetic assay with clinical risk as determined by PCPT RC. In this model the epigenetic assay proved again to be the strongest, significant predictor (OR = 3.17; P <0.001), whereas the PCPT RC score was borderline significant (P = 0.0825). The AUC was 0.652, which is a significant improvement of patient risk assessment through the addition of the epigenetic assay compared to the PCPT RC alone (P <0.001).

Interestingly, when the methylation intensity was added to the final model, it provided a significant, independent contribution (OR = 4.85; P = 0.002) to the other risk factors in the model (i.e., the epigenetic assay [OR = 2.46; P <0.001], the presence of atypia [OR = 2.80; P <0.001], PSA [OR = 1.89; P = 0.068], and the presence of HGPIN [OR = 1.20; P = 0.406]). With an AUC of 0.695, the effect on the overall accuracy of the risk stratification model was limited, but significant (P = 0.044) (Figure 3).

Risk Score for GS ≥ 7 PCa

With the increased focus on high-grade PCa, but the lack of pathological grades in this cohort, men with clinically low-grade cancers (GS ≤6) were excluded from the analysis. The remaining cohort consisted of 624 men with a negative repeat biopsy and 67 men with a GS ≥7 repeat biopsy. DRE was again found to be the least reliable predictor, resulting in a final model consisting of the epigenetic assay, age, pathology, and PSA, which reached an AUC of 0.707. The performance of the PCPT RC also improved in this setting, with an AUC of 0.609; however, this was still significantly lower than that of the model including the epigenetic assay (P = 0.007). The addition of DNA-methylation intensity to the model was again good for a minor, but significant increase of the AUC to 0.729 (P = 0.017) (Figure 4).

DISCUSSION

This meta-analysis shows a consistent performance of an epigenetic assay, demonstrating an overall high NPV of 89.2%, which is a significant improvement compared to current clinical practice. The NPV increases further to 96.0% for high-grade PCa, also significantly

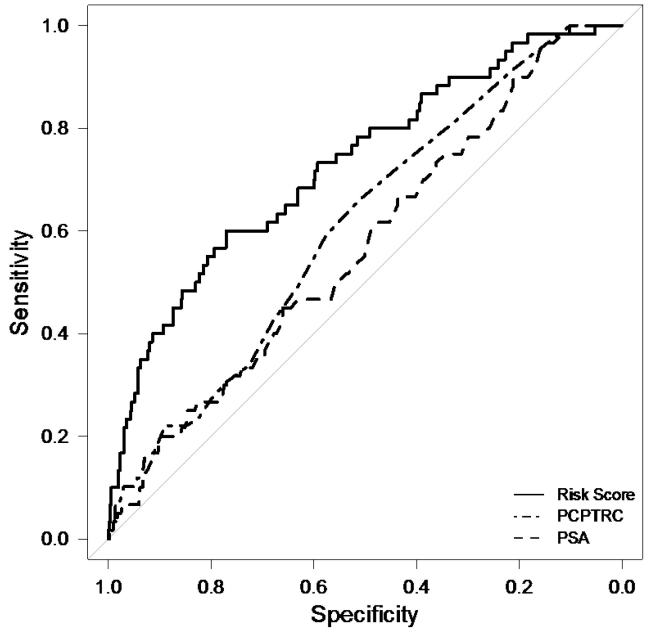

FIG. 4. Receiver operating characteristic curves for PSA, PCPT RC, and a risk score as determined with an epigenetics-based multimodal risk assessment model for high-risk prostate cancer. PSA, prostate-specific antigen; PCPTRC, Prostate Cancer Prevention Trial Risk Calculator.

improving over the current standard of care and giving an increased confidence of a negative biopsy result. Multimodal risk assessment approaches are becoming more valuable, integrating several information sources to better guide patients. The addition of epigenetic profiles can play an important role, as clearly illustrated by the enhanced performance of and its central role in such models. Indeed, all models were highly dependent on the inclusion of the epigenetic assay for the most optimal patient risk stratification. Notably, a model that included the epigenetic assay, pathology (HGPIN, atypia or benign) of the cancer-negative index biopsy specimen and PSA was a significant improvement over the commonly used PCPT RC and would significantly enhance patient selection for repeat biopsy.

DNA-methylation, and the epigenetic assay in particular, proved to be the most important component to stratify men for the risk of PCa in general, but also specifically for high-grade disease. PSA played a somewhat ambiguous role when used as sole predictor; however, it

proved to have some added value in multivariate models (i.e., when used in conjunction with other risk factors). DRE, on the other hand, never played a role of significance in any of these models, nor was it a good predictor by itself. The only risk factor that came close to the epigenetic assay in terms of raw performance was the presence of atypia in a cancer-negative index biopsy specimen.

Multimodal risk stratification models also proved to be highly valuable in the context of high-grade, clinically significant cancer, resulting again in significantly improved patient risk stratification relative to the current clinical standard, especially when factoring in a patient's epigenetic profile. In addition, a risk score was built that does not only incorporate the binary result of the epigenetic assay, but also the intensity of the DNA-methylation signals in a patient's individual core biopsy tissues. Because a clear correlation was observed between the DNA-methylation levels and the clinical tumor grade, this further improved risk stratification, especially in terms of detecting occult high-grade PCa in patients with a negative biopsy.

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DISCUSSION

Howley, Boston: In looking for biomarkers have you evaluated whether circulating DNA is at all predictive and if in these cases?

Partin, Baltimore: Two of my colleagues at Hopkins, Drs. Bill Nelson and Vasan Yegnasubramanian, are looking at the circulating DNA in the bloodstream with a very similar approach. Bill Nelson actually discovered GST-PI methylation. They have found very promising results which would eliminate us having to biopsy to get the material to do the test. I see that as a being very promising in the future.

Zeidel, Boston: Very nice talk. From what I understand about prostate biopsies, which is very little, that there is a lot of inter-observed variability in what they are seeing on the slide in terms of grading and it would be very helpful to think about looking at the longer term outcome. I also understand it there is a lot of difference in outcomes for example in someone with a Gleason score of 7. Many of them do just fine and many of them do terribly and it's very hard right now to predict who is who. So the question of whether this can be extended and long-term follow-up up of these patients could be engineered, I think would be extremely helpful in this disease. Any comment about that?

Partin, Baltimore: That's a great comment and there are also at least four other commercially available products now trying to investigate this with the analysis of gene mutations, addition and loss of genes. These panels are quite expensive. They run as high as $3,000 for some of the tests. This test is about a $100–$500 even if your insurance doesn't pay for it. I agree with you. We do need to do that. It would be wonderful to do that from the blood as opposed to having actually undergo the biopsy. A lot of people are working on that.

Mushlin, New York City: You know I have also found this talk very interesting. I think we obviously need guidance and help in assisting these patients in making probably one of the most difficult clinical decisions that we have out there today, namely what to do about prostate cancer. Your focus on the group with biopsies I think is a useful one. I applaud you for looking at negative predictive value, which I think is where the money is, so to speak, in this. As you know, negative predictive value is very much a function of the probability of the disease as well as the sensitivity of the diagnostic test. You have a test that has modest sensitivity, 64%, so what's driving the negative predictive value is mostly the probability of the disease. That is going to vary by the population in which

the diagnostic test is applied. I guess my question is, what can be done to enhance the sensitivity to really try to achieve better negative predictive value? Have you thought about the opportunity to move along the ROC curve to maximize sensitivity at the loss of increased specificity? Or diminished specificity?

Partin, Baltimore: Well, you are absolutely correct on the statistical arguments. If I just went and biopsied random men, I could get the negative predictive value to maybe 99%. But the fact is that these were men that had high-risk characteristics coming in. They had high PSAs, they had high-grade prostatic intraepithelial neoplasia (PIN) on a previous biopsy, and we are concerned that they may actually harbor prostate cancer. These three genes are not going to get sensitivity up to what you and I would like but some combination of genes in the future may. We don't have that gene for prostate cancer now. If you just use PSA alone as a biomarker and you take it out to the community, which thank God we aren't doing anymore, you get negative predictive value of 95% to 97% because most men's PSAs are less than 1. It's that group where the PSA is around 4, where we all really need to focus on and try to help them make the decisions. Your statistic arguments are right; we can't beat that. That's just the way of Mother Nature.

Mushlin, New York City: You might be able to if you think about opportunities to move around the ROC curve, you know?

Partin, Baltimore: I don't think these three genes are going to do that because, you know, my mentors taught me you find what you look for. We were looking for negative predictive value and those 3 out of the 300 that they looked at pushed it to the highest limit there. Thank you though.

Wolf, Boston: I just want to move back a step and I was wondering if the degree of methylation in blood, urine, or semen in patients with a high PSA could be used to predict those who are going to have a negative biopsy and then not do the biopsy?

Partin, Baltimore: Ultimately, yes. We don't know that answer right now. Semen isn't very good. We went through this idea over a decade ago. Men don't want to come to the clinic and give you a semen specimen, and a lot of the men we were evaluating for prostate cancer were at an age where it is very difficult to even collect one.

Wolf, Boston: It's easier to give semen than have a biopsy.

Partin, Baltimore: True, it is. Saliva would be even better if we could do a somatic or germline type of mutation in your cheek swab and it could tell us if you're going to develop prostate cancer. But you are absolutely correct, imaging now has come a long way. We use MRIs now. No man gets a repeat prostate biopsy, at least in our institution, without first getting an MRI. I think we would break the economic bank if every man got an MRI. But when someone who was high risk has a negative biopsy and you are thinking of repeating the biopsy, a MRI guiding biopsy is absolutely fantastic.

Oates, Nashville: In terms of an optimal biomarker, it would be important to know whether the methylation of DNA is secondary to histone modifications whether it's a primary if driven by the mechanisms for methylation.

Partin, Baltimore: You might get contradictory views, I am a surgeon but I think that if Bill Nelson, who discovered this, were here, he would probably agree with you. They have a very complex understanding of why these genes are getting methylated. The genes are turned off because they are not needed all the time. It's a gene that is needed when you have toxins floating around in your liver. I don't know the answer to that. As one of my mentors in my MD, PhD program, Paul Delay, would say, as he took off his glasses, "That's an excellent question and the answer may be known but not by me."